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(54) Title: FIBRIN-CELL SUSPENSION FOR CONSTRUCTION OF NEW TISSUE

(57) Abstract

A method for creating new tissue is described wherein cells are suspended in cryoprecipitate or fibrinogen (jointly referred to herein as "fibrinogen") which is clotted by exposure to thrombin or other serine esterase. In the preferred embodiment, the cells and fibrinogen are autologous, derived by biopsy and cryoprecipitation of a plasma sample, respectively. The method is particularly useful for creation of new cartilage in the repair of defects such as worn or torn joint linings or construction of ears and noses. The method is also particularly useful where tissue such as endothelial tissue or cartilage is used to oblate a structure, such as fallopian tubes or the vas deferens, or in the repair of defects such as incomplete closure of structures, for example, the heart. Examples demonstrate formation of new cartilage in mice.

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FIBRIN-CELL SUSPENSION FOR CONSTRUCTION OF NEW TISSUE

Background of the Invention

The present invention is generally in the area of medical treatments, and specifically relates to an method for creating new tissue, especially cartilage, bone and endothelial tissue.

Cartilage is a specialized type of dense connective tissue consisting of cells embedded in a matrix. There are several kinds of cartilage. Translucent cartilage having a homogeneous matrix containing collagenous fibers is found in articular cartilage, in costal cartilages, in the septum of the nose, in larynx and trachea. Articular cartilage is hyaline cartilage covering the articular surfaces of bones. Costal cartilage connects the true ribs and the sternum. Fibrous cartilage contains collagen fibers. Yellow cartilage is a network of elastic fibers holding cartilage cells which is primarily found in the epiglottis, the external ear, and the auditory tube.

Damage to cartilage and bone by disease, such as arthritis, or trauma is a major cause of physical deformity and debilitation. In medicine today, the primary therapy for loss of cartilage is replacement with a prosthetic material, such as silicone for cosmetic repairs, or metal alloys for joint relinement. Placement of permanent prostheses is commonly associated with significant loss of underlying tissue and bone without recovery of the full function allowed by the original cartilage, as well as the irritating presence of a foreign body. Other long term problems associated with a permanent foreign body can include infection, erosion and instability. Very little has ever been actually used clinically to replace

the cartilage overlaying bone surfaces. Although International patent application WO 89/00413 published 26 January 1989 describes the use of a glycosaminogycan prosthetic meniscus for implantation in the knee that allows ingrowth of adjoining tissues, this technology has not been utilized clinically. Despite several preparations being tested to stimulate growth and repair of the remaining cells, in most cases repair to injuries is made surgically. Patients suffering from degeneration of cartilage can only turn to drugs having analgesic or antiinflammatory properties, or compounds such as hyaluronic acid, for relief.

The lack of truly biocompatible, functional prostheses can have profound and tragic effects for those individuals who have lost noses or ears due to burns or trauma, such as car accidents or war. The best surgeons can do for these patients is to carve a piece of cartilage out of a piece of lower rib to approximate the necessary contours and insert it into a pocket of skin in the area where the nose or ear is missing.

In the past, bone has been replaced using actual segments of sterilized bone or bone powder or porous surgical steel seeded with bone cells which were then implanted. An example of a process using bone powder and a hydrated collagen lattice is U.S. Patent No. 4,485,097 to Bell. An example of the implantation of a seeded porous metal prosthesis is U.S. Patent No. 4,553,272 to Mears. The success of these implants has been limited, in part because of the non-degradable nature of the cell supports. Hattori, J. Jpn. Orthop. Assoc. 64(9), 824-834 (1990), describes the implantation of fibrin glue and bone morphogenic protein (BMP) to induce osteo-chondrogenesis, but with only mixed success. The author concluded that the fibrin glue

was effective in combination with BMP to induce osteo-chondrogenesis.

To date, the growth of new cartilage or bone from either transplantation of autologous or allogeneic chondrocytes, osteocytes, or fibroblasts has been largely unsuccessful other than using the method of U.S. Patent No. 5,041,238 to Vacanti, et al. Microscopic islands of cartilage formation have been demonstrated histologically *in vivo* by implanting recombinant bone morphogenic protein, as reported by J.M. Wozney, et al., Science, 242, 1528-1534, (December 16, 1988). Limited success has been achieved in making neocartilage using free autogenous grafts of perichondrial flaps, as described by J. Upton, Plastic and Reconstructive Surgery, 68(2), 166-174, (August 1981). Pitman, et al., reported that fibrin glue and mussel adhesive protein had been tried as adhesives for an implant made by preparing a slurry of chondrocytes in collagen, pouring the slurry onto a fibrillar collagen matrix, and polymerizing the collagen. A problem had been observed with stabilization of these implants. In addition to the high variability between surgeons, the graft was easily displaced. The results demonstrated that the muscle adhesive was better as an adhesive than the fibrin glue, in large part because the fibrin sets up almost instantaneously and any movement irreversibly breaks the bonds. Ohlsen and Nordin, Scand. J. Plastic Reconstructive Surg. 20:259-271 (1986), reported on the potential usefulness in rabbits of perichondrial grafts (dissected from the ears) for reconstruction of the larynx. Fontana, et al., Aesthetic Plastic Surgery 15:237-240 (1991), reported that one could implant cartilage chips bound with fibrin glue in rhinoplasty but do

not describe long term efficacy or whether the cartilage is eventually degraded.

U.S. Patent No. 5,041,138 to Vacanti, et al., discloses the use of an open synthetic polymeric matrix which is seeded with cells for implantation into a patient to create new cartilage. A fibrous structure is preferred to allow maximum surface area for attachment of cells at the same time as allowing for sufficient diffusion of nutrients and gases to maintain cell viability in the absence of vascularization. In contrast to the work reported by others using implantation of differentiated tissue, such as the cartilage chips of Fontana, et al. or implantation of sterilized bone, dissociated cells are seeded onto matrices which are then implanted and evolve into new tissue. This technique has now been used extensively in animals and a human, with promising results. This technique is limited however, by the availability of suitable polymeric support materials.

Despite these promising results, it would be advantageous if one could have a method for culturing new tissue, such as cartilage, bone, or endothelial tissue which did not require implantation of exogenous materials, with the possible exception of non-autologous cells.

It is therefore an object of the present invention to provide a method and means for making a completely autologous, natural implant for formation of new tissue.

It is a further object of the present invention to provide a method and means for making an implant for formation of new cartilage or bone wherein the implant has osteo-chondrogenesis activity.

It is a further object of the present invention to provide methods for performing oblative surgery using closed surgical procedures and autologous, endogenous materials.

Summary of the Invention

A method for creating new tissue is described wherein cells are suspended in cryoprecipitate or fibrinogen (jointly referred to herein as "fibrinogen") which is clotted by exposure to thrombin or other serine esterase. In the preferred embodiment, the cells and fibrinogen are autologous, derived by biopsy and cryoprecipitation of a plasma sample, respectively. Other protein matrices can be substituted for the fibrin, where the protein has similar characteristics and can be crosslinked easily by addition of an agent such as calcium.

The method is particularly useful for creation of new cartilage in the repair of defects such as worn or torn joint linings or construction of ears and noses. The method is also particularly useful where tissue such as endothelial tissue or cartilage is used to oblate a structure, such as fallopian tubes or the vas deferens, or in the repair of defects such as incomplete closure of structures, for example, the heart. Examples demonstrate formation of new cartilage in mice.

Detailed Description of the Invention

The method described herein generally consists of making a suspension of dissociated cells that are to be implanted to form new tissue with an aqueous solution of a crosslinkable endogenous protein, implanting the suspension into the site where tissue is to be formed, and crosslinking the protein. In the preferred

embodiment, the protein solution is cryoprecipitate or fibrinogen derived from the patient in which the tissue is to be implanted, which is clotted with human thrombin, and the cells are autologous cells derived from the patient by biopsy.

Fibrinogen

In the preferred embodiment, cryoprecipitate is prepared from a plasma sample obtained directly from the patient into which the cell suspension is to be implanted. One unit of human blood consists of approximately 350 to 450 mls, which is preferably collected in ACD (citric acid-dextrose) anticoagulant, although other acceptable anticoagulants can be used such as ethylene diamine tetraacetate. The red blood cells are removed by centrifugation or filtration, and the separated plasma chilled at 4°C until cryoprecipitate is formed, typically about three days. Cryoprecipitate consists predominantly of fibrinogen. Fresh frozen plasma can also be used.

Purified fibrinogen is also available from commercial suppliers such as Sigma Chemical Co., Baxter Diagnostics, and Ortho Pharmaceuticals.

As used herein, the term "fibrinogen" is intended to encompass either cryoprecipitate or purified fibrinogen, unless specifically stated otherwise.

Other materials that can be used as a source of fibrinogen besides cryoprecipitate, fresh frozen plasma, and purified fibrinogen, include factor VIII concentrate, platelet concentrate, and platelet rich plasma.

Proteins other than fibrinogen can be substituted for the fibrinogen where the protein can be prepared and crosslinked using a physiologically acceptable crosslinker such as calcium. An advantage of the fibrinogen is that it

is readily obtained in sufficient quantities merely by drawing blood from the intended recipient so that there is no problem with patient rejection of the implant.

Cells

In the preferred embodiment, cells of the same species and preferably immunological profile are obtained by biopsy, either from the patient or a close relative, which are then grown to confluence in culture using standard techniques known for cell culture and used as needed. If cells that may elicit an immune reaction are used, such as human chondrocytes from an immunologically distinct individual, then the recipient can be immunosuppressed as needed, for example, using a schedule of steroids and other immunosuppressant drugs such as cyclosporine A, Imuran, or prednisone, using the dosages recommended by the manufacturer for immunosuppression of transplants, and/or the cells irradiated prior to transplantation. However, in the most preferred embodiment, the cells are autologous. Cultured cells of some cell types are available from sources such as the American Type Culture Collection, Rockville, MD. Only normal, non-transformed cells which exhibit contact inhibition characteristic of normal cells should be implanted.

Examples of cell types that can be implanted to form cartilage include chondrocytes, subchondral cells and fibroblasts. Examples of cell types that can be implanted to form bone include osteocytes, periosteocytes and fibroblasts, under appropriate conditions. Examples of parenchymal cell types include many types of cells present in tissues such as intestine, pancreas, liver. Examples of other cell types that can be implanted include muscle cells, Dermafibroblasts,

mesenchymal cells and cells forming tendons and ligaments.

Cells obtained by biopsy are harvested and cultured, passaging as necessary to remove contaminating non-chondrocytes. For example, cartilage can be obtained from another site in the patient or from autopsy, using for example, cartilage obtained from joints or rib regions. The cartilage is sterilized, for example, by washing in Povidone-Iodine 10% solution (Betadine, Purdue Frederick Co., Norwalk, Conn.), then, under sterile conditions, the muscle attachments are dissected from the underlying bone to expose the joint surfaces. The cartilage from the articulating surfaces of the joint is then sharply dissected from the underlying bone. The cartilage is cut into pieces with dimensions of less than 5 mm per side and washed twice in Phosphate Buffered Saline (PBS) with electrolytes and adjusted to neutral pH. The minced cartilage is then incubated at 37°C in a solution of 0.2% clostridial collagenase (Worthington CLS II, 140 U/mg) and agitated overnight as described by Klagsbrun, (Methods in Enzymology, Vol. VIII). This suspension is then filtered using a 153 μg nylon sieve (Tetko, Elmford, N.Y. 10523). The cells are washed with PBS, counted using a hemocytometer, and concentrated to yield a chondrocyte concentration of between approximately 10^7 to 10^8 cells/cc, most preferably 5×10^7 cells/cc.

The isolated chondrocytes can be cultured in Hamm's F-12 culture media and 10% fetal calf serum with L-glutamine (292 μg/cc), penicillin (100 U/cc), streptomycin (100 μg/cc) and ascorbic acid (5 μg/cc), at 37°C.

Precursor cells of chondrocytes can also be used in place of the chondrocytes. An example

is fibroblasts which differentiate to form chondrocytes. As described herein, the term "chondrocytes" includes chondrocyte precursor cells.

Other cell types are similarly dispersed. For example, muscle cells can be obtained by mincing small detrusor muscle fragments to approximately 0.5 mm diameter and using these as explants in 100 mm tissue culture dishes containing 10 mL of DMEM supplemented with 10% fetal calf serum. Outgrowth is routinely observed at 72 hr after explants are placed in culture. When cultures are 80% confluent, the cells were trypsinized and passaged. Cell populations highly enriched in elongated, striated cells are routinely obtained using this method.

Cells are preferably suspended in nutrient media, such as Ham F12 or MEM 199, or in saline or buffer, for mixing with fibrinogen and implantation.

Thrombin

The fibrinogen is clotted by exposure to an enzyme such as thrombin in the presence of physiological calcium immediately prior to or at the time of implantation. Additional crosslinking is obtained by exposure to factor XIIIa. Thrombin is commercially available from the same suppliers as purified human fibrinogen, for example, Parke-Davis or Johnson and Johnson; it appears to be irrelevant whether it is human or bovine. In the preferred embodiment, 10,000 units of thrombin is added to the cryoprecipitate from one unit of whole blood. Other serine esterases are known to those skilled in the art, such as factor Xa, which can be used instead of thrombin to cleave the fibrinogen into fibrin. Other esterases such as factor XIIIa can also be used. Thrombin and factor XIIIa cleave

fibrinogen or fibrin monomers, respectively, during the normal clotting process.

Preparation of Cell-Fibrinogen Suspension

In the preferred embodiment, the cells are mixed with the fibrinogen, thrombin and optionally factor XIIIa, in the presence of calcium (typically, 80 mM), to form a cell-hydrogel matrix which is implanted. In the case of chondrocytes, the fibrinogen from one unit of blood is mixed with approximately 25 x 10^6 cells/ml. The cell-fibrin/fibrinogen or cryoprecipitate mixture is then implanted at the site where tissue is to be created. As the cells proliferate *in situ*, new tissue forms to the extent of the implanted material, i.e., the fibrinogen-cell mixture, but not beyond.

Applications

The method and materials described herein can be used in a wide variety of applications, in which tissue formation is effective to treat the disorder or defect.

Cartilage formation

In one embodiment cartilage is formed that serves as a joint lining. Typically, the cell solution is implanted into the joint, and cartilage forms over a period of a few weeks. The joint is immobilized while new cartilage is formed.

In another embodiment, cartilage is formed that serves as a structure such as a nose or ear. In this case, the fibrinogen-cell suspension is implanted at the site and immediately shaped, preferably simultaneously or immediately prior to crosslinking.

In other embodiments, tissue which can be cartilage or other cell types is formed by implantation of the appropriate fibrinogen-cell suspension at the site where tissue formation is

desired. In some cases this will be to obstruct a lumen or close an opening resulting from a congenital defect, trauma, or disease.

As reported by Atala et al, "Injectable alginate seeded with chondrocyte as a potential treatment for vesicoureteral reflux" J. Urol. 150:745 (1993), alginate, a biodegradable polymer, embedded with chondrocytes, can serve as a synthetic substrate for the injectable delivery and maintenance of cartilage architecture in humans. A biopsy of the symphysis pubis can be easily and quickly performed using a biopsy gun followed by chondrocyte processing and endoscopic injection of the autologous chondrocyte/alginate suspension for the treatment reflux. As described herein, the alginate can be replaced with fibrinogen clotted with thrombin. It is preferable to implant rather than inject the fibrinogen-cell mixture, however, and the degradation profile of the totally endogenous fibrinogen is distinct from that of alginate or synthetic polymers.

The studies showed that chondrocytes can be easily harvested and combined with a matrix *in vitro*, implanted cystoscopically and the elastic cartilage tissue formed is able to correct vesicoureteral reflux without any evidence of obstruction. The resulting autologous cartilage is non-antigenic, non-migratory, and volume stable. Since the chondrocyte are autologous, this method of treatment does not require FDA approval. The procedure can be performed under 15 minutes, with a short period of a mask anesthetic, in the outpatient unit, without any need for a hospital stay. Neither vesical nor perivesical drainage is required. Since the whole procedure is done endoscopically and the bladder is not entered surgically, there is no postoperative discomfort.

The patient can return to a normal level of activity almost immediately.

Other applications are performed in a similar manner. The cells to be implanted are obtained, for example, by biopsy from the patient who is also to be the recipient, the cells are dissociated and proliferated, a unit of blood is drawn from the patient and cryoprecipitate made, the cryoprecipitate is mixed with the cells and thrombin in the presence of calcium, and the mixture implanted at the site where tissue is to be formed: in a lumen, in a joint lining, in the mesentery or adjacent an adequate blood supply or highly vascularized area, optionally with a portocaval shunt in the case of hepatocytes and other cells benefitting from the addition of hepatotropic factors. For example, the cell-matrix suspensions could be used to obliterate fistula related to the sinus tract, nasal-cutaneous, bronchopleural and intraabdominal fistulae, by endoscopic injection of the suspension into the cavity. Heart valves, trachea, inner ear structures, and implants could be formed by using molds seeded with cells in a fibrin matrix allowed to grow *in vitro*, then implanted subcutaneously.

The present invention will be further understood by reference to the following non-limiting examples.

Example 1: Preparation of Fibrinogen-Cell Matrix.

Cryo-Precipitation

Whole blood was collected in a standard blood bag containing citrate-phosphate dextrose anticoagulant solution. It was then centrifuged at 4°C at 4200 rpm for 6 minutes. The plasma (210 to 300 ml) was frozen at less than -18°C for 18 hours prior to further processing. The fresh-frozen

plasma (FFP) was thawed in a 4°C circulating water bath for one hour, mixed, and removed after two hours when completely thawed. The thawed plasma was then recycled through the freezing and thawing process by storing it at less than -18°C for at least 18 hours before thawing again in the 4°C water bath. The twice frozen and thawed plasma was centrifuged at 4°C at 4200 rpm for 12 minutes. Following centrifugation the bag containing the cryoprecipitated fibrinogen was inverted and the plasma drained into the attached transfer bag, leaving 3 to 5 cc of concentrated fibrinogen. The product for clinical use routinely contains 39.8 to 58.9 mg/ml fibrinogen. The autologous fibrinogen concentrate can be frozen at less than -18°C for storage of up to one year from the collection date of the whole blood. The range of concentrations obtainable is from 20 to 77 mg/ml.

Prior to surgical application, the autologous fibrinogen concentration is thawed at 30 to 37°C in a water bath. The thawed fibrinogen concentrate may be stored at 20 to 24°C for up to six hours before preparing the fibrin matrix.

Preparation of Fibrin Matrix

Four ml of calcium chloride solution (40 mM/liter) was added to a vial of thrombin (1000 U) and swirled until completely dissolved, for a final concentration of 250 U/ml. Equal volumes of calcium chloride/thrombin and fibrinogen concentrate were mixed together simultaneously according to the surgical protocol for application of the fibrin suspension. Fibrinogen gels into a fibrin clot within 20 to 30 seconds with a thrombin mixture concentrate of 250 U/cc, which is preferred for injection. Higher concentrations of thrombin (500 to 1000 U/Ml), are preferred for implantation, since the fibrin starts to gel in less than 15

seconds. Cells are added to the cryoprecipitated fibrinogen before adding the thrombin.

Other Methods for Creating Fibrinogen

One cc of 3.8% sodium citrate solution per tube was added to 9 cc of human blood and centrifuged for 10 minutes at 3200 rpm, producing fibrogen. 500 NIH units of thrombin diluted with 5 cc of H_2O and 20 drops of amino caproic acid, a fibrinolysis inhibitor, were then added.

36 cc of human blood was drawn and divided into four tubes each containing 9 cc. One cc of 10% sodium citrate solution was added to each tube and the tube was centrifuged at 3200 rpm for 10 minutes. The plasma was syphoned off and cooled over ice (0°C for 10 minutes). Eight volumes percent of ethanol (usually 0.4 cc) was added to each tube, the tube twirled and fibrinogen was observed to precipitate. The tube was centrifuged at room temperature for another 10 minutes at 32000 rpm and the supernatant syphoned off. Between about 0.5 to 1.0 cc of fibrinogen remained in each tube, which was kept at 37°C. 1000 NIH units of thrombin diluted with 1.5 cc of amino caproic solution (20 mg/cc sterile H_2O) was then added to the fibrinogen.

Example 2: Neocartilage formation in a fibrin glue matrix.

Materials and Methods

Isolation of cells: Bovine chondrocytes were isolated by enzymatic digestion from the glenohumeral joint surface of calf forelimbs. The isolated cells were combined with 1 ml of cryoprecipitate to create a final cell density of 12.5×10^6 cells/ml. Bovine thrombin was added to the mixture to produce a polymerizing gel. The resulting fibrin glue-chondrocyte admixture was placed subcutaneously in eighteen sites in nude mice. Separate control implants consisting of

chondrocyte suspension or fibrin glue alone were also implanted. All specimens were weighed and processed for histological, total DNA, and glycoaminoglycan (GAG) analysis to demonstrate production of neocartilage.

Histology and histomorphometry: Specimens from each concentration and time point were collected. One half of each specimen was placed in 10% buffered formalin for histological preparation (the remaining half was used for GAG and DNA analysis described below). The tissue was fixed for a minimum of 24 hours and dehydrated in increasing concentrations of ethanol and embedded in paraffin. Five micron sections were made and stained with hematoxylin and eosin, toluidine blue, safranin-O. Cartilage samples from donor articular cartilage processed in an identical manner served as one control. The histological sections were examined in a blinded fashion by three independent investigators and scored.

Glycosaminoglycan content: Total glycosaminoglycan (GAG) content of the cell/polymer constructs was determined using a dimethylmethylene blue (DMB) dye method. DMB stock solution was made using DMB, NaCl, Glycine, Na azide, 1 N HCl, and water. The samples were digested with papain (125 mg/ml buffer of 0.1 M NaH_2PO_4, 5 mM EDTA, 5 mM cysteine HCl at pH 7.0 overnight at 60°C) before adding the dye. GAG was to be reported as a percent of wet weight.

Total DNA content: Total DNA content was determined using one-fourth of an implant specimen using the bisbenzimidazol fluorescent dye method. The dye (Hoechst 33258) was used in a concentration of 0.2 mg/ml in a 0.01 M Tris, 1 mM EDTA and 0.1 M NaCl. The samples were digested with papain (125 mg/ml in buffer of 0.1 M NaH_2PO_4, 5 mM EDTA, 5 mM

cysteine HCl at pH 7.0 overnight at 60°C) before adding the dye. A spectrofluorometer was used to estimate the fluorescence of the digested samples, emission was measured at 400-550 nm for an excitation wavelength of 365 nm. Uncultured chondrocytes in a solution of calf thymus DNA was used to generate standardized curves.

At harvest, the fibrin glue-chondrocyte implant specimens had the gross appearance of cartilage; whereas the control specimens were entirely absorbed and unretrievable. The presence of cartilage was confirmed histological by a blinded histopathological observer. DNA/GAG analysis confirmed the presence of actively proliferating chondrocytes with production of a well-formed cartilagenous matrix. The mean GAG content in the neomatrix was 473 μg/ml (SD ± 240). The mean amount of GAG in relation to wet weight was 1.9% (SD ± 0.8%). The mean DNA content was estimated at 40.7 μg/ml (SD ± 12.4).

We claim:

1. A method for treating anatomical defects comprising administering to a patient in need of treatment thereof at the site of the defect a suspension of cells in a fibrinogen solution and adding thrombin and calcium in an amount effective to form a fibrin matrix incorporating the cells at the site of the defect.

2. The method of claim 1 wherein the cells are selected from the group consisting of chondrocytes, subchondral cells, fibroblasts, osteocytes, periosteocytes, parenchymal cells, muscle cells, Dermafibroblasts, mesenchymal cells and cells forming tendons and ligaments.

3. The method of claim 1 wherein the fibrinogen is selected from the group consisting of cryoprecipitate, purified fibrinogen, fresh frozen plasma, factor VIII concentrate, platelet concentrate, and platelet rich plasma.

4. The method of claim 1 wherein the cells are isolated from the patient.

5. The method of claim 1 comprising administering the cells and fibrinogen into a joint.

6. The method of claim 1 comprising administering the cells and fibrinogen subcutaneously and shaping the cell-fibrin mixture to form a structure.

7. The method of claim 1 wherein the site is in proximity with the mesentery or other highly vascularized region.

8. The method of claim 1 wherein the cell-fibrinogen mixture is administered to correct vesicoureteral reflux.

9. The method of claim 1 wherein the patient has incontinence and the cell-fibrinogen mixture is administered to correct the condition.

10. The method of claim 1 wherein the defect is in the thoracic region.

11. The method of claim 1 wherein the defect is in the upper gastrointestinal tract.

12. The method of claim 1 wherein the cell-fibrin mixture is implanted into a lumen.

13. The method of claim 1 wherein the cell-fibrin mixture is implanted to correct a fistula or other opening in tissue.

14. The method of claim 1 wherein the cell-fibrin mixture is implanted to form inner ear structures.

15. An isolated cell-fibrinogen suspension in combination with thrombin and calcium in an amount effective to form a cell-fibrin matrix structure following implantation into a patient to form tissue.

16. The suspension of claim 15 wherein the cells are selected from the group consisting of chondrocytes, subchondral cells, fibroblasts, osteocytes, periosteocytes, parenchymal cells, muscle cells, Dermafibroblasts, mesenchymal cells and cells forming tendons and ligaments.

17. The suspension of claim 15 wherein the fibrinogen is selected from the group consisting of cryoprecipitate, purified fibrinogen, fresh frozen plasma, factor VIII concentrate, platelet concentrate, and platelet rich plasma.

18. A method for making a cell-fibrin structure comprising obtaining cells from a patient, suspending the cells in a fibrinogen solution, adding thrombin and calcium to the cell-fibrinogen mixture in an amount effective to form a cell-fibrin matrix structure after implantation into a patient, and implanting the fibrinogen suspension into a patient to form new tissue.

19. The method of claim 18 further comprising shaping the matrix after implantation.

20. The method of claim 18 wherein the fibrinogen is formed from cyroprecipitate.

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PC /US 95/09374

A. CLASSIFICATION OF SUBJECT ER
IPC 6 A61L25/00 27/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	EP,A,0 339 607 (ITAY S.) 2 November 1989 see column 3, line 21 - line 41; claims 1,7,8	1-6, 14-19 8-11,20
X	GB,A,2 137 209 (RAMOT UNIVERSITY) 3 October 1984 see page 2, line 76 - line 82; claims 1-11	1-6, 14-19
X Y	WO,A,94 02182 (OPPERBAS HOLDING) 3 February 1994 see claims 1,2,6,8	15 20
X	WO,A,92 22312 (WADSTRÖM J.) 23 December 1992 see claims 1,27	1

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[X] Further documents are listed in the continuation of box C.

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3 November 1995	17. 11. 95
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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 95/09374

C.(Continuation) DOCUMENTS [redacted] IDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,92 09301 (THE AMERICAN NATIONAL RED CROSS) 11 June 1992 see claim 1	1
P,Y	WO,A,94 25080 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 10 November 1994	8-11

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
US 95/ 09374

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-14
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 1-14 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte· onal Application No
PCT/US 95/09374

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